55. (amended) A method for determining whether an agent is capable of modulating the degradation of a target 3' polyadenylated messenger RNA sequence in the absence of deadenylation comprising

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(A) providing a a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate, a source of ATP; and an exogenous target 3' polyadenylated messenger RNA sequence; in the presence of a nucleotide triphosphate;

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(B) introducing said agent into said cytoplasmic extract; and

(C) monitoring the degradation of said target 3' polyadenylated messenger RNA sequence in said extract thereby determining whether said agent is capable of modulating said degradation.

Remarks

Claims 1, 2, 4-6, and 9-55 were pending in the subject application. Claims 1, 4-6, 9-10, 14-15, 17, 21, 23, 25-29, 31, 33, 35-42, 44, 46-48, 51, 53, and 55 have been amended. Claim 56 has been added. Claims 12, 13, 16, 22, and 34 have been cancelled without prejudice. Consequently, claims 1, 2, 4-6, 9-11, 14-15, 17-21, 23-33 and 35-56, as amended, are under consideration.

The claims have been amended to better define the claimed invention, to clarify claim language, and to make claims consistent with previous amendments. Support for the amendments adding 3' polyadenylated messenger RNA to claim 1 and dependent claims is found in the specification on page 16, lines 5-7, page 27, lines 11-14, and page 39, lines 11-13, and in addition in the Examples. Support for the amendments to claims 9 and 10 is found on page 5, lines 10-22, Support for the amendments to claims 17, 23, and 35 is found in the specification on page 6, lines 8-12. Support for the amendment to claims 31 and 44 is found in these claims as filed. Claims 12, 13, 16, 22, and 34 have been cancelled without prejudice as redundant in view of the amendments to claim 1. Support for new claim 56 is found in claim 51 as filed. No issue of new matter is raised.

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Formalities

Claims 10, 14, 25, 36, and 37 have been objected to for several grammatical details. The Examiner's recommendations as to correction have been implemented in the present Amendment, accordingly it is respectfully requested that the objections be withdrawn.

35 U.S.C. §112 Rejections:

§112, First Paragraph:

All that is required under §112, first paragraph, is that applicants show a skilled person how to make and use their claimed invention. This has been done in the subject application. These rejections are respectfully traversed

Claims 30-32 and 43-45 have been rejected under §112, first paragraph, as allegedly not enabled for a C-rich element binding protein. C-rich element binding proteins are defined in the specification on page 7, lines 5-7, page 8, lines 7-8, and page 20, lines 24-25 through page 21, line 1 as RNA binding molecules which are RNA stability modifiers and that bind to C-rich elements on RNA, such as those found in the messenger RNAs of globin, collagen, lipoxygenase, and tyrosine hydroxylase.

This is sufficient information for a skilled person to obtain a C-rich element binding protein and use the system of claim 21 to determine whether an agent being tested can modulate its activity. C-rich binding elements and proteins which bind to them were well known in the art at the time the above application was filed.

As confirmation, enclosed as Exhibits 1-5 are references disclosing C-rich binding elements (Chkheidze et al. 1999 Mol. Cell. Biol. 19:4572-4581; Russell et al. Mol. Cell. Biol. 1998 18:2173-2183; Holcik and Leibhaber 1997 PNAS (USA) 94:2410-2414; Weiss and Liebhaber 1995 Mol. Cell Biol. 15:2457-2465; and Wang, et al. 1995 Mol.Cell. Biol. 15:1769-1777. See also Kiledjian et al. 1995 EMBO J. 14L4357-4364). These references show trans-acting factors which bind to C-rich elements, which are C-rich element binding proteins. Wang in particular shows how to obtain a C-rich binding protein.

Thus it is clear that the specification and what is known in the art provides sufficient information for a skilled person to obtain C rich element binding proteins and to use them in the claimed invention.

Therefore, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 30-32 and 43-45 under §112, first paragraph.

Claims 48-50 have been rejected under §112, first paragraph, as allegedly not enabled for modulating cell growth or cell differentiation. This rejection is respectfully traversed.

These claims are directed to a method for identifying an agent capable of modifying cell growth or differentiation by determining the agent's ability to modulate the stability of an mRNA involved in modulation of cell growth or differentiation, in particular transformation or immune dysregulation.

The Examiner alleges that in order to practice the invention of these claims, it would be necessary to first identify an mRNA involved in cell growth or differentiation, then determine whether modulating the stability of the mRNA affects growth or differentiation, then assay for changes in stability of the mRNA, then correlate the changes in stability to changes in cell growth or differentiation.

In contrast, the point of this aspect of the claimed invention is that *if* a candidate agent modifies stability of a target mRNA involved in growth or differentiation, then the agent *is* one which modifies cell growth or differentiation. The system of this invention can determine this. It is not necessary to determine whether modulation affects growth or differentiation, or to assay for changes in stability, or to correlate the changes in stability to growth or differentiation. Using the system to determine that the agent modulate stability of the target mRNA is sufficient to determine that the agent affects growth or differentiation. The point of the claimed method is that using it makes it unecessary to determine whether modulating the stability of the mRNA affects growth or differentiation, then assay for changes in stability of the mRNA, then correlate the changes in stability to changes in cell growth or differentiation.

That the method uses mRNA involved in cell growth or differentiation is not a basis for §112 rejection. A skilled practitioner would be able to obtain such mRNA. Proteins involved in

cell growth and cell differentiation, and their subcategories of transformation and immune regulation, are well known and the genes encoding them have been sequenced, so that isolating or synthesizing the corresponding mRNA would present no undue difficulties to the skilled person.

All that §112 requires is that the specification show a skilled person how to make and use the claimed invention. Methods for using the system of this invention, for example to determine whether an agent modulates the stability of target mRNA are described in the specification throughout, such as on pages 20-31 and pages 33-34, and on pages 37-57.

Thus, the claimed method meets the requirements of §112, first paragraph, and, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 48-50 under §112, first paragraph.

Claims 31 and 44 have been rejected under §112, first paragraph, as allegedly not enabled for "tristetrapolin". The Examiner states that "tristetrapolin" cannot be found in several databases. Applicants submit that "tristetrapolin" is a typographical error for the protein "trisetraprolin". Enclosed as Exhibit 6 is a review article. Wilusz, et al. 2001 Nat. Rev. Mol. Cell Biol. 2:237-46, at 240; (see also for example Cytokines Online Pathfinder Encyclopaedia; Brennan and Steitz 2001 Cell. Mol. Life Sci. 58:266-277 at 269, column 2) which shows that that tristetraprolin is an ARE binding protein grouped with HuB, HuC, HuR, the ELAV family, AUF, and others. Note that this is how "tristetrapolin" is described in the subject specification. Tristetraprolin is intended. Since "tristetraprolin" is a well known protein and presents no §112 issues, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 31 and 44 under §112, first paragraph.

§112, Second Paragraph:

Claims 9-11, 15-17, and 21-50 have been rejected under §112, second paragraph as allegedly indefinite. It is respectfully submitted that these rejections do not apply to the claims, and the claims as amended.

The Examiner alleges that dependent claims 9-11 do not further limit claim 1, since claim 1 is drawn to a system and claims 9-11 are allegedly drawn to method steps. Applicants do not agree that claims 9-11 do not limit claim 1. However, in order to forward prosecution and clarify claim language these claims have been amended and are drawn to specific components of the system of claim 1 and not to steps.

The Examiner alleges that claims 10 and 25 are unclear in their recitation of antibodies to proteins that bind polyadenylate, polyadenylate, and the combination thereof. Claim 25 does not recite this phrase, and claim 10 has been amended to clarify the subject matter as 1. antibodies to proteins which... 2. polyadenylate, and 3. the combination of antibodies to proteins which..., and polyadenylate.

Claims 10, 14, 25, and 36 have been amended to change "the combination" to "a combination", in accordance with the Examiner's recommendations.

The Examiner alleges that "a ligand" in claims 15, 26, and 37 is unclear. However, when taken in context the meaning of a ligand is clear to a skilled person. On page 29, paragraph 2 of the specification, the detectably labelled RNA of these claims is described. Ligands are given as one example of various labelling agents such as enzymes, fluorophores, radioisotopes, etc., and biotin is given as an example of a ligand. In this context, it is clear that ligand means a molecule such as biotin which is bound to the entity to be labelled (here the RNA) and to which another labelled molecule then binds to complete the labelling, such as when biotin is bound to a nucleic acid, then labelling completed by allowing streptavidin to bind to the biotin. Therefore, "ligand" does not present an issue under §112, second paragraph.

Claims 16, 17, 22, 23, 34, and 35 limit the system of claim 1 to exogenously added nucleoside triphosphate, specifically ATP. Claim 1 as previously amended includes a source of ATP. Therefore in order to avoid redundancy, claims 16, 22, and 34 have been cancelled without prejudice and claims 17, 23, and 35 have been amended to further limit the source of ATP to an exogenously added ATP. It is respectfully submitted that the remaining claims are therefore in order.

The Examiner points out that claim 21 recites "turnover" with no antecedent basis. However, claim 21 was amended in the Amendment and Response under 37 C.F.R. 1.111 filed

October 26, 2000 to recite "deadenylation and degradation" instead of "turnover". Thus, this part of the rejection is moot.

The Examiner points out that claims 31 and 44 recite "member of the ELAV family" followed by a list of proteins, and states that it is not clear whether the list of proteins following "member of the ELAV family" are examples of ELAV family members or other additional proteins. The other proteins are not members of the ELAV family but are additional proteins (see Exhibits 8-11). This is clear in the claim format, where language "...consisting of a member of the ELAV family; AUF1; tristetrapolin; AUH; TIA;...and AUFB" is used, and each protein is separated by a semicolon. This usage indicates that each of the referenced items after the "consisting of" and followed by a semicolon is a separate item. Therefore, it is respectfully submitted that these claims present no §112 indefiniteness issue. Nor do claims 32 and 45 present any such issue. These claims depend on claims 31 and 44, and each claim limits its parent claim by reciting specific members of the ELAV family.

The Examiner points out that claims 31 and 44 each recite "tristetrapolin". As demonstrated above, by "tristetrapolin" is meant the well known protein "tristetraprolin". The claims have been amended accordingly.

Claim 47 has been rejected as allegedly unclear with regard to the addition of nucleoside triphosphate to the claimed system which includes ATP. This claim has been amended to delete nucleoside triphosphate, since the claimed system was previously amended to include ATP.

Claim 51 has been rejected for the language "isolating a molecule suspected of participating in" a reaction. Although this language clearly indicates that the method is one for screening molecules to determine whether they participate in the reaction, in the interests of furthering prosecution the claim has been amended to further clarify the language. In addition, it is pointed out that claim 51 uses the phrase "target RNA" with no antecedent. The antecedent is the system of claim 1 referred to in step (A), which includes a target RNA. The claim has been amended to expressly incorporate "target RNA".

Claim 55 has been rejected for allegedly failing to recite an identification step. In the interests of furthering prosecution, claim 55 has been amended to clarify this point.

Prior Art Rejections

The undersigned would like to thank the Examiner for the courtesy of a telephone interview on December 11, 2001. In the interview, Dr. Wilusz, the Examiner, and the undersigned discussed the pending prior art rejections. The substance of the interview is discussed below.

Rejections under 35 U.S.C. §103

Claims 1, 5-6, 9-17, 21-29, 33-42, 46-47, and 51-55 have been rejected under 35 U.S.C. §103(a) over Bernstein (Mol. Cell. Biol. 9:659-670), Levy I (J. Biol. Chem. 271:2746-2753), Levy II (J. Biol. Chem. 273:6417-6423) and Shapiro (Biochemistry 8:659-670) in combination. This rejection is respectfully traversed with regard to the pending claims.

The invention of claims 1, 5-6, 9-17, 21-29, 33-42, 46-47, and 51-55 is a system for recapitulating regulated RNA deadenylation and degradation which combines a target RNA (a messenger RNA which includes the usual 3' poly-A sequence) with an S100 cytoplasmic extract (centrifuged at 100,000 x g for 1 hour) supernatant depleted of polyadenylate-binding activity, and ATP. Also part of the invention are methods for using the system to identify agents which modulate the stability of the target mRNA, and a kit for this purpose.

The Examiner states that Bernstein teaches cellular extracts depleted of polyadenylate binding proteins (PABPs) combined with target RNAs, and modulating agents which are PABPs, polyA, and anti-PABP antibodies. Bernstein does not teach using an S100 cytoplasmic extract supernatant or added ATP. Levy I is cited to add the S100 cytoplasmic extract in which mRNA binding proteins which modulate degradation allegedly are found. The Examiner further states that it would be obvious to use the Levy I cytoplasmic extract in the Bernstein method, because Levy II provides motivation by allegedly teaching the desirability of a system which recapitulates regulated expression and degradation of RNA. Since neither Bernstein or Levy teach added ATP, Shapiro is added as allegedly teaching that ATP and UTP stimulate deadenylating enzyme activity, and combined on the basis that it would therefore be obvious to add ATP to the Bernstein, Levy I, and Levy II methods. Shapiro does not itself disclose a system.

This rejection was discussed in the telephone interview mentioned above. In the interview, applicants pointed out that neither Bernstein or the Levy articles disclose mRNA degradation and deadenylation. What these references teach is simply RNA degradation. The difference is important, because there are RNA nucleases that can degrade mRNA, but are not necessarily involved in regulated mRNA turnover. Regulated mRNA turnover plays an important role in cellular metabolism, for example by determining the stability of a given mRNA and consequently the effective life span of proteins. Nonspecific mRNA degradation is not relevant to this regulated process. In regulated mRNA turnover, a polyadenylate-specific RNA nuclease deadenylates the mRNA (i.e. removes the 3' poly-A tail). After deadenylation, exonucleases are activated which rapidly degrade the remainder of the now deadenylated mRNA. It is this two-step process which must be reproduced in order to analyze the actual physiological stability of a given mRNA.

The system of this invention recapitulates actual regulated RNA turnover, where deadenylase-mediated deadenylation occurs before general degradation, and general degradation is rapid and without detectable intermediates, and there are no nonspecific RNA nucleases. The cited references do not disclose or suggest any such system whether alone or in combination. Deadenylation of 3' polyadenylated mRNA is not disclosed or suggested by the above references because none of them disclose 3' polyadenylated mRNA. There is no deadenylase activity disclosed by these references because there is no polyadenylase substrate for the deadenylase to act on. Accordingly these references do not provide any means to obtain regulated RNA turnover. Thus the claimed system is not rendered obvious by the references whether alone or in combination.

The Examiner pointed out that the lack of 3' polyadenylated mRNA could overcome the pending rejections if the claims were directed to 3' polyadenylated mRNA, and if applicants could demonstrate that the Bernstein and both Levy references do not disclose 3' polyadenylated mRNA. The claims have been amended accordingly, and the requested demonstration is made below.

With regard to Bernstein, the RNA's disclosed are not 3' polyadenylated (no "poly-A tail"). Bernstein uses two mRNAs, human H4 histone mRNA, and human beta-globin mRNA

(see page 660, column 1. last paragraph). Human H4 histone mRNA is well known to lack the poly-A tail. (see, for example, McLaren et al. 1997 Mol. Cell. Biol. 17:3028-3036, page 3028, column 2, second paragraph, enclosed as Exhibit 7; see also Ross 1995 Microbiol. Rev. 59:423-450, page 428, column 2, line 1). Therefore the histone mRNA disclosed by Bernstein has no poly-A tail. With regard to the beta-globin mRNA, as is shown below, the process by which this mRNA was cloned inevitably leaves bases derived from restriction endonuclease sites 3' to the poly-A tail. Since deadenylase needs a poly-A substrate to act in the absense of poly-A RNA no deadenylase activity can occur, and RNA degradation, not regulated RNA turnover, is the result (see Korner, et al. 1998 EMBO J. 17:5427-5437 enclosed as Exhibit 8; see also Ford, et al. 1999 Genes Dev. 13:188-201).

In more detail, the human beta globin mRNA used in Bernstein is described in Bernstein's reference 48, which is Peltz, et al. 1987 J. Biol Chem. 262:9382-9388 enclosed herewith as Exhibit 9. The Peltz beta globin construct is described as follows on page 9383, column 2, first paragraph:

"A human beta globin cDNA clone, pSPkbeta/c (kindly provided by K. Lang and R. Spritz, Dept of Genetics, University of Wisconsin) was cleaved with either *Eco*RI or *Hin*dIII. Transcription of the EcoRI cleaved plasmid generated a 415-nucleotide, **nonpolyadenylated** RNA that terminates near the end of the translated region. **The *Hin*dIII cleaved pSPkbeta/c DNA generates a full-length mRNA substrate with an approximately 40 nucleotide 3' terminal poly(A) tract plus any bases transcribed from the *Hin*dIIIoverhang."** (emphasis added)

What this means is that the beta globin mRNA used by Bernstein did not end in AAAAAAAA, but in AAAAAAAACGA, the last nucleotides being generated by the HindIII site. As shown above, deadenylase requires a polyadenylase substrate to act. Deadenylase therefore could not degrade the 3' CG at the end of the Bernstein mRNA. Accordingly, since neither of the Bernstein mRNA's have a 3' poly(A) tail, deadenylation of 3' polyadenylated mRNA is not disclosed, suggested, or enabled since no deadenylase is possible in the absence of its substrate.

In addition, Bernstein uses different cell-derived material from that of the claimed invention. The cell material in the claimed system is the supernatant from centrifuged cytoplasm (S100 cytoplasmic extract). This is an extract of the whole cytoplasm. In contrast, Bernstein a high-salt extract of polysomes, called ribosomal salt wash (RSW). The RSW is also called a

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supernatant (see page 660, column 1, first paragraph "...the supernatant or ribosomal salt wash (RSW) fraction is harvested."). However, this RSW supernatant is not the same material as the supernatant from an S100 cytoplasmic extract. Bernstein cites Ross and Kobs 1986 J. Mol. Biol. 188:579-593 enclosed herewith as Exhibit 10 for the method by which RSW supernatant is obtained. As can be seen on page 580, column 2, cells were processed to obtain a polysomal pellet, and these polysomes were then resuspended and mashed in a homogenizer to solubilize them, forming a polysome preparation. In contrast, as can be seen for example in Levy I, page 2747, column 1, first full paragraph, cells were centrifuged to pellet nuclei, and the supernatant from this step was then centrifuged at 100,000 x g to provide the S100 cytoplasmic extract. It is the supernatant from such an S100 extract that is used in the claimed method. In contrast with Bernstein, this supernatant is not a polysome preparation but a different material. Thus Bernstein does not teach using the supernatant from an S100 extract. This is yet another reason why Bernstein does not disclose or suggest the claimed system.

With regard to both Levy I and Levy II, the mRNAs disclosed also do not have poly-A tails (Levy II uses the same methods as Levy I, citing Levy I for the RNA degradation assays from which the text which follows is excerpted). As is the case in Bernstein , the process by which this mRNA was cloned inevitably leaves bases derived from restriction endonuclease sites 3' to the poly-A tail. Again, since deadenylase needs a poly-A substrate to act in the absense of poly-A RNA no deadenylase activity can occur, and RNA degradation, not regulated RNA turnover, is the result

In more detail, Levy I states on page 2747, first column, paragraph 1 that

"[^{32}P]CTP-labeled, capped, and polyadenylated transcripts were synthesized *in vitro*(18) . The *Eco*RI site of pSP64 poly(A) (Promega) was transformed into an *Ase*I restriction enzyme site by filling in *Eco*RI-digested pSP64 poly(A) with the Klenow fragment and then blunt-end ligating the vector. Restriction fragments containing the 3`-UTR of VEGF derived from clone 11.4 (12) were cloned into the multiple cloning site of this modified pSP64 vector. A series of deletions were made from the 3` end of these sequences using unique restriction sites in the VEGF 3`-UTR. Digestion of these plasmids with *Ase*I-generated DNA templates containing a poly(dT) sequence that was transcribed into a 30-base long poly(A) tail at the 3` end."

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However, the 3' end of these constructs does not have a poly(A) tail but has the sequence CCGAATA. Thus when the pSP64 vector is processed as described above, the 3' end of the resulting mRNA has at its 3' end CCGAATTA rather than a poly(A) tail. This can be seen clearly in the flow chart attached hereto as Exhibit 11 derived from the above description and depicting the nucleotide sequences of the relevent restriction sites at each step of the Levy I construction process, showing how CCGAATTA ends up at the 3' end of the RNA transcribed from the Levy I construct. (the sequence and map of pSP64 are also part of Exhibit 11 and are available to the public at the Promega website).

Since, as shown above, deadenylase requires a polyadenylase substrate to act, and therefore could not degrade the 3' CG at the end of the Levy I and II mRNA, deadenylation of 3' polyadenylated mRNA is not disclosed, suggested, or enabled since no deadenylase is possible in the absence of its substrate.

Moreover, Levy does not disclose or suggest depleting a cellular extract of polyadenylase binding proteins (PABP), which depletion is an important part of the claimed method.

The Examiner has combined the S100 extract of Levy I and II with the PABP depletion shown in Bernstein, in an attempt to reconstruct the claimed invention. It is well-settled that to support a §103 rejection combining references there must be basis provided in the references to make these combinations and basis to expect that the combination would "work" (see for example In re Fine, 5 USPQ2d 1596 (Fed. Cir. 1988); In re Vaeck, 20 USPQ2d 1438 (Fed. Cir. 1991)). No such a basis is present here, in fact the references teach against such combination. One would not combine Levy with Bernstein, because polysomes or a high salt extract of polysomes (RSW) is a very different material than an S100 cytoplasmic extract. Clearly, there are no polysomes in the S100 extract. One would not perform a procedure that expressly excludes polysomes using polysomes or polysome-derived materials. Therefore, there is no basis to combine Levy I and Levy II with Bernstein.

Moreover, even in combination, the claimed invention is not suggested by the references, because as demonstrated above none of the relevant references discloses 3' polyadenylated RNA. In the absence of poly-A mRNA, there can be no regulated turnover of mRNA, because polyadenylation, the necessary first step, cannot occur.

Based on the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 1, 5-6, 9-17, 21-29, 33-42, 46-47, and 51-55 under §103.

Claim 4 has been rejected under 35 U.S.C. §103(a) over Bernstein (Mol. Cell. Biol. 9:659-670), Levy (J. Bio. Chem. 271:2746-2753), Levy (J. Biol. Chem. 273:6417-6423) and Shapiro (Biochemistry 8:659-670) in combination, and further in view of Krikorian (J. Virology 65:112-122). This rejection is respectfully traversed with regard to the pending claims.

Claim 4 is directed to the system of claim 1 where the cytoplasmic extract is obtained from HeLa cells or a T cell line. Krikorian has been cited to disclose HeLa cells (cytoplasmic extracts from which were obtained by low speed centrifugation and used in RNA degradation reaction without depletion of PABPs - in contrast to the system of claim 4 which uses S100 cytoplasmic extracts containing no polysomes and further depleted of PABPs). Krikorian does not disclose or suggest the claimed invention. And, as shown above, neither do the remaining references taken individually or taken in combination. In addition, it is noted that none of the references disclose a T cell line. Thus, Krikorian in combination with the other cited references does not disclose or suggest the system of claim 4. Therefore, the Examiner is respectfully requested to reconsider and withdraw the rejection of claim 4 under §103.

Claims 2, 30-32, and 43-45 have been rejected under 35 U.S.C. §103(a) over Bernstein (Mol. Cell. Biol. 9:659-670), Levy (J. Bio. Chem. 271:2746-2753), Levy (J. Biol. Chem. 273:6417-6423) and Shapiro (Biochemistry 8:659-670) in combination and further in view of Myer (EMBO 16:2130-2139) and Chen (Mol. Cell Biol. 15:5777-5788). This rejection is respectfully traversed with regard to the pending claims.

The claims are directed to the system and methods of this invention where mRNA deadenylation and degradation are mediated by AU-rich element or C-rich element binding proteins.

Myer is cited to disclose such proteins and their involvement in destabilization of mRNA, and describes the isolation of one such protein. Chen is cited to disclose that such proteins are modified by agents which modify deadenylation. Neither reference discloses a system such as

that claimed. Since as shown the remaining references do not disclose or suggest the claimed invention, the addition of Chen and Myer do not remedy the other references.

Based on the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 2, 30-32, and 43-45 under §103.

<u>Claims 18-20 and 53</u> have been rejected under 35 U.S.C. §103(a) over Bernstein (Mol. Cell. Biol. 9:659-670), Levy (J. Bio. Chem. 271:2746-2753), Levy (J. Biol. Chem. 273:6417-6423) and Shapiro (Biochemistry 8:659-670) in combination and further in view of Fellig (Arch. Biochem. Biophys. 85:313-316). This rejection is respectfully traversed with regard to the pending claims.

Claims 18-20 and 53 are directed to the system and methods of this invention with the addition of a reaction enhancer such as polyvinyl alcohol. Fellig discloses that sulfate-containing polymers such as sulfated polyvinyl inhibit pancreatic ribonuclease. Fellig does not disclose or suggest a system or methods as claimed. It is noted that Fellig discloses sulfated polymers and the claimed invention does not use sulfated polymers. In addition, the reaction enhancers of the claimed invention *stimulate* RNA degradation (see specification, page 31, paragraph 2). Since Fellig discloses *inhibition* of ribonuclease, this would suggest against using a polymer to enhance a degradation reaction. Since as shown the remaining references do not disclose or suggest the claimed invention, combination with Fellig also fails to suggest the claimed invention.

Based on the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 18-20 and 53 under §103.

Enclosed herewith is a Petition for a two month extension of time.

Attached hereto is a page captioned "Version with markings to show changes made", which shows the amended claims with additions underlined and deletions bracketed.

Applicants respectfully solicit allowance of the subject application.

Respectfully submitted,

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March 1, 2002

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Serial. No. 09/320,609
Filed May 26, 1999

Version with markings to show changes made

Please amend the claims by deleting bracketed material and inserting underlined material as follows:

1. (three times amended) An *in*[-]*vitro* system that recapitulates regulated RNA deadenylation and degradation of an exogenously[-]added preselected target 3' polyadenylated messenger RNA sequence comprising

i) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate;

ii) a source of ATP; and

iii) an exogenous target 3' polyadenylated messenger RNA sequence.

4. (amended) The system of claim 3 wherein said [cell] cytoplasmic extract is obtained from a cell line selected from the group consisting of HeLa cells and a T cell line.

5. (amended) The system of claim 1 wherein said [cell] cytoplasmic extract is prepared from cells comprising foreign nucleic acid.

6. (amended) The system of claim 1 wherein said [cell] cytoplasmic extract is prepared from cells which are infected, stably transfected, or transiently transfected.

9. (amended) The system of claim 1 wherein said [cell] cytoplasmic extract [depleted of activity of proteins that bind polyadenylate] is [prepared by a method] selected from the group consisting of:

(a) [addition to said system of] a cytoplasmic extract which contains polyadenylate competitor RNA;

(b) a cytoplasmic extract which contains a material that sequesters [ration of] proteins that bind polyadenylate;

(c) [addition of] a cytoplasmic extract which contains a proteinase that inactivates a protein that bind to polyadenylate; and

(d) [addition of] a cytoplasmic extract which contains an agent that prevents the interaction between polyadenylate and an endogenous macromolecule that binds to polyadenylate.

10. (amended) The system of claim 9 wherein [said sequestration of proteins that bind polyadenylate is achieved by treatment of said extract with an] the material that [depletes macromolecules that bind polyadenylate] sequesters proteins that bind polyadenylate is selected from the group consisting of 1. antibodies to proteins that bind polyadenylate, 2. polyadenylate, and 3. a [the] combination [thereof] of antibodies to proteins that bind polyadenylate, and polyadenylate.

14. (amended) The system of claim 1 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled 3' polyadenylated messenger target RNA sequence, a labeled 3' polyadenylated messenger target RNA sequence, and a [the] combination thereof.

15. (amended) The system of claim 14 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety is selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

17. (amended) The system of claim 1 [16] wherein said [nucleotide triphosphate is] source of ATP is exogenous.

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21. (amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence comprising

(A) providin[e]g the system of claim 1;

(B) introducing said agent into said system;

(C) determining the extent of deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence; and

(D) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence.

23. (amended) The method of claim 1 [22] wherein said [nucleotide triphosphate is] source of ATP is exogenous.

25. (amended) The method of claim 21 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled target 3' polyadenylated messenger RNA sequence, a labeled target 3' polyadenylated messenger RNA sequence, and a [the] combination thereof.

26. (amended) The method of claim 25 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety is selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

27. (amended) The method of claim 21 wherein said monitoring the extent of turnover of said target 3' polyadenylated messenger RNA sequence comprises determining the extent of degradation of said labeled target 3' polyadenylated messenger RNA.

28. (amended) The method of claim 21 wherein said modulating the stability of a target 3' polyadenylated messenger RNA sequence increases the stability of said target RNA sequence.

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29 (amended) The method of claim 21 wherein said modulating the stability of a target 3' polyadenylated messenger RNA sequence decreases the stability of said RNA sequence.

31. The method of claim 30 wherein said AU rich element binding protein is selected from the group consisting of a member of the ELAV protein family; AUF1; [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B.

33. (amended) A method for identifying an agent capable of modulating the stability of a target 3' polyadenylated messenger RNA sequence in the presence of an exogenously added RNA stability modifier comprising

(a) providing the system of claim 1;

(b) introducing said RNA stability modifier into said system;

(c) introducing said agent into said system;

(d) determining the extent of turnover of said target 3' polyadenylated messenger RNA sequence; and

(e) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target 3' polyadenylated messenger RNA sequence in the presence of said exogenously added RNA stability modifier.

35. (amended) The method of claim 1 [34] wherein said [nucleotide triphosphate is] source of ATP is exogenous.

36. (amended) The method of claim 33 wherein said target 3' polyadenylated messenger RNA sequence is selected from the group consisting of an unlabeled target 3' polyadenylated messenger RNA sequence, a labeled target 3' polyadenylated messenger RNA sequence, and a [the]combination thereof.

37. (amended) The method of claim 36 wherein said labeled target 3' polyadenylated messenger RNA sequence is labeled with a moiety [is] selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

38. (twice amended) The method of claim 36 wherein said determining the extent of deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence comprises determining the extent of degradation of said labeled target 3' polyadenylated messenger RNA.

39. (amended) The method of claim 33 wherein said RNA stability modifier increases the stability of said target 3' polyadenylated messenger RNA sequence.

40. (amended) The method of claim 39 wherein said agent decreases the stability of said target 3' polyadenylated messenger RNA sequence increased by said RNA stability modifier.

41. (amended) The method of claim 33 wherein said RNA stability modifier decreases the stability of said target 3' polyadenylated messenger RNA sequence.

42. (amended) The method of claim 41 wherein said agent increases the stability of said target 3' polyadenylated messenger RNA sequence decreased by said RNA stability modifier.

44. (amended) The method of claim 43 wherein said AU rich element binding protein is selected from the group consisting of a member of the ELAV protein family; AUF1; [tristetrapolin] tristetraprolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP A1; AU-A; and AU-B.

46. (three times amended) A method for identifying an agent capable of modulating regulated deadenylation of a target 3' polyadenylated messenger RNA sequence comprising

(A) providing a system that recapitulates regulated RNA deadenylation of an exogenously[-]added preselected target 3' polyadenylated messenger RNA sequence comprising

i) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate;

ii) said target 3' polyadenylated messenger RNA sequence;

(B) introducing said agent into said system;

(C) monitoring the deadenylation of said target 3' polyadenylated messenger RNA sequence in said system; and

(D) identifying an agent able to modulate the extent of said deadenylation as capable of modulating the regulated deadenylation of said target 3' polyadenylated messenger RNA sequence.

47. (amended) A method for identifying an agent capable of modulating the deadenylation and degradation of a target 3' polyadenylated messenger RNA sequence comprising

(A) providing the system of claim 1 [in the presence of a nucleotide triphosphate];

(B) introducing said agent into said system;

(C) monitoring the deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence in said system; and

(D) identifying an agent able to modulate the extent of said deadenylation and degradation as capable of modulating the deadenylation and degradation of said target 3' polyadenylated messenger RNA sequence.

48. (amended) A method for identifying an agent capable of modulating cell growth or cell differentiation in a mammal comprising determining the ability of said agent to modulate the stability of a target 3' polyadenylated messenger RNA sequence involved in the modulation of cell growth or differentiation in accordance with claim 19.

51. (amended) A method for [identifying, characterizing or isolating] <u>determining whether</u> an endogenous molecule [suspected of] participat[ing]<u>es</u> in the deadenylation or degradation of RNA or regulation thereof comprising

(A) providing the system of claim 1 <u>containing target 3' polyadenylated messenger RNA</u>;

(B) introducing said <u>endogenous molecule</u> [protein suspected of participating in the regulation of RNA turnover] into said system; <u>and</u>

(C) monitoring the stability of said target <u>3' polyadenylated messenger</u> RNA sequence in said system[;] <u>thereby determining whether said endogenous molecule is capable of modulating said regulation</u>.

[(D) [identifying, characterizing or isolating said endogenous molecule able to modulate said deadenylation or degradation as capable of participating in the deadenylation or degradation of RNA or regulation thereof.]

53. (three times amended) A kit for monitoring the stability of a preselected exogenous target <u>3' polyadenylated messenger</u> RNA sequence under conditions capable of recapitulating regulated RNA deadenylation and degradation, said kit comprising:

(a) a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation, said extract depleted of activity of proteins that bind polyadenylate;

(b) other reagents; and

(c) directions for use of said kit.

55. (amended) A method for [identifying] <u>determining whether</u> an agent <u>is</u> capable of modulating the degradation <u>of</u> a target <u>3' polyadenylated messenger</u> RNA sequence in the absence of deadenylation comprising

(A) providing a [cell extract] a <u>cytoplasmic</u> extract <u>supernatant from a 100,000 x g, 1 hour centrifugation isolated from eukaryotic cells or tissues, said extract depleted of activity of proteins that bind polyadenylate, a source of ATP; and an exogenous target 3' polyadenylated messenger RNA sequence;</u> in the presence of a nucleotide triphosphate;

(B) introducing said agent into said [cell] cytoplasmic extract; and

(C) monitoring the degradation of said target 3' polyadenylated messenger RNA sequence in said extract thereby determining whether said agent is capable of modulating said degradation.

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